# Effect of Pork Belly Composition and Nitrite Level on Nitrosamine Formation in Fried Bacon

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A study was conducted to determine the effect of compositional factors (fat, moisture, protein) on nitrosamine formation in bacon prepared from matched pairs of pork bellies cut into thirds. The compositional factors varied significantly ($p = 0.05$) from section to section within the same side but did not vary from side to side within the same section of matched pair. Both *N*-nitrosopyrrolidine and *N*-nitrosodimethylamine were most highly correlated with residual and added nitrite and to a lesser degree with the compositional factors.

*N*-Nitrosopyrrolidine (NPYR) (Crosby et al., 1972; Fazio et al., 1973) and to a lesser extent *N*-nitrosodimethylamine (NDMA) have been found consistently in fried bacon at the ppb level (Sen et al., 1973; Wasserman et al., 1978), while these and other nitrosamines have been found only sporadically in other cured meat products (Fiddler et al., 1975; Gough et al., 1977; Havery et al., 1976; Panalaks et al., 1974; Wasserman et al., 1972). The presence of nitrosamines in this product may be due primarily to the high cooking temperatures which would favor the reaction of residual nitrite with nitrosatable amine compounds. Several authors have demonstrated the importance of cooking temperature and method on the NPYR content of bacon (Herring, 1973; Pensabene et al., 1974; Wasserman et al., 1978). However, neither ham nor breakfast beef subjected to similar cooking conditions produces detectable concentrations of NPYR (Fiddler et al., 1974). This suggests that bacon may be unique in containing more readily nitrosatable precursor(s). Fiddler et al. (1974), Patterson et al. (1976), and Mottram et al. (1977) have associated NPYR formation with bacon adipose tissue and not with lean tissue. We have observed that bacon having a high fat to lean tissue ratio and yielding more rendered fat tended to have a higher concentration of NPYR than bacon having a lower fat to lean ratio. In our studies on bacon and nitrosamine formation, proper sampling has always been a problem because of the great variability of fat, moisture, and protein content of the green bellies used for processing. Stiffler et al. (1975) reported average lean differences as high as 10% at ten different anatomical positions. Schroder and Rust (1974) reported average fat contents ranging from 30 to 70% at 32 belly positions and concluded that there was as much compositional variation within the same belly as among different bellies. No significant difference in composition was observed between similar sections of paired bellies from the same carcass.

We are reporting here a new sampling scheme for investigating nitrosamine formation and for determining the effect of compositional factors on nitrosamine formation in fried bacon.

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## EXPERIMENTAL SECTION

**Bacon Processing.** Skinned matched pork bellies were purchased from a local supplier within 1 day of slaughter and stored for 1 week in a cooler at 1 °C. The bellies were cut into thirds (brisket, center, and flank sections) and pumped to approximately 10% of their green weight to achieve added target levels of 1.5% sodium chloride, 0.5% sugar, 0.3% sodium tripolyphosphate, and 200 ppm sodium nitrite (actual range 170–260 ppm). The pumped bellies were stored in polyethylene bags at 1 °C for 20–22 h, then processed in a smokehouse with the following schedule of increasing heat and controlled humidity: 1 h dry bulb (DB) 38 °C, wet bulb (WB) 0 °C; 1 h DB 50 °C, WB 0 °C; 3 h DB 57 °C, WB 47 °C. A medium to heavy smoke was introduced after 2 h of drying. The finished bacon reached an average internal temperature of 53 °C (128 °F) after 5 h. The bacon sections were placed in polyethylene bags and stored at 1 °C for 18 h.

**Bacon Sampling and Frying.** Each section of the belly was ground and thoroughly mixed three times through a $^1/_8$ in. plate prior to analyses. A 350-g representative sample of the comminuted bacon was fried for 6 min, with turning every 2 min, at a calibrated temperature of 177 °C (350 °F) in a preheated Presto Teflon-coated electric frying pan. Both the edible portion and rendered drippings were retained for nitrosamine analyses.

**Bacon Analysis.** *a. Nitrite.* Residual nitrite values were determined before frying by the Griess-Saltzman reaction in the procedure described by R. N. Fiddler (1977). The added nitrite values were calculated.

*b. Fat, Moisture, Protein.* Fat determinations were made by the Foss-let solvent extraction procedure described by Pettinati and Swift (1975). Moisture determinations followed the oven drying method (Official Methods of Analysis, 1975a), and protein analysis was carried out by the Kjeldahl procedure (Official Methods of Analysis, 1975b).

**Nitrosamine Analysis.** *a. Fried Bacon.* A 25-g fried bacon sample, to which 1 mL of *N*-nitrosomethylethylamine internal standard (0.5 $\mu$g/mL of $CH_2Cl_2$ solution)

Table I. Mean Values for Composition of Bacon

| pair | side | fat, %[a] [1] brisket | center | flank | moisture, %[2] brisket | center | flank | protein, %[3] brisket | center | flank |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | left | 47.4 | 54.3 | 42.4 | 38.9 | 35.5 | 41.6 | 10.0 | 8.6 | 9.1 |
| | right | 48.7 | 55.2 | 47.5 | 39.7 | 33.5 | 39.4 | 9.6 | 9.0 | 10.3 |
| 2 | left | 52.1 | 55.4 | 46.1 | 38.7 | 37.7 | 42.9 | 9.8 | 9.1 | 10.9 |
| | right | 53.2 | 54.5 | 45.2 | 37.9 | 36.6 | 41.2 | 10.0 | 9.8 | 10.5 |
| 3 | left | 51.3 | 58.8 | 47.3 | 36.3 | 34.4 | 38.1 | 8.6 | 6.8 | 10.1 |
| | right | 50.5 | 54.8 | 45.1 | 36.0 | 33.1 | 41.3 | 9.7 | 8.4 | 10.4 |
| 4 | left | 49.5 | 57.2 | 51.8 | 36.0 | 31.1 | 34.7 | 10.1 | 8.2 | 9.6 |
| | right | 48.7 | 57.8 | 52.6 | 37.7 | 32.4 | 34.0 | 10.0 | 8.0 | 9.7 |

[a] Percent SD between duplicate determinations: [1]fat ± 0.59, [2]moisture ± 0.46, [3]protein ± 0.32.

was added and mixed, was placed in a Virtis flask; 80 mL of distilled water and 10 mL of $CH_2Cl_2$ were added. The mixture was homogenized for 15 min in a Virtis blender, transferred to a polypropylene bottle, and centrifuged 10 min at 5000 rpm, and the supernatant was transferred to a round-bottom flask containing 75 mL of 5 N NaOH and 8 g of $Ba(OH)_2$. The solids in the centrifuged bottle were shaken with 25 mL of distilled water for 1 min and recentrifuged for 5 min. The supernatants were combined and distilled until all the aqueous distillate was collected. After addition of 25 g of NaCl and 5 mL of 6 N HCl, the distillate was extracted three times with 125 mL of $CH_2Cl_2$. The combined extracts were washed with 50 mL of 6 N HCl and then with 5 N NaOH. The $CH_2Cl_2$ extract was dried by passage through anhydrous $Na_2SO_4$ and concentrated to 1.0 mL in a Kuderna-Danish apparatus. The average recovery of the internal standard was 78%.

*b. Cooked-Out Fat.* Nitrosamines in the fat drippings were isolated and separated by the method of White et al. (1974). The resulting $CH_2Cl_2$ extract was concentrated (ca. 1.0 mL) and applied to a water-cooled column (10 mm × 7 cm) containing silica gel 60 (70–230 mesh, Brockmann activity 2–3, E. Merck, Darmstadt, Germany), washed with 150 mL of $CH_2Cl_2$–pentane (25:75, v/v), and eluted with 125 mL of ether–$CH_2Cl_2$ (30:70, v/v). The eluate was then concentrated as above.

*c. Determination.* The volatile nitrosamines were quantitated by GLC–thermal energy analyzer under conditions similar to those described by Fine et al. (1975) and confirmed by GLC–high-resolution mass spectrometric analysis (1:12 000) under conditions previously reported (Pensabene et al., 1974).

NOTE: Precaution should be exercised in the handling of nitrosamines since they are potential carcinogens.

## RESULTS AND DISCUSSION

Investigations were carried out to determine the effect of sample locations, i.e., side and section, on the composition (fat, moisture, and protein) of bacon derived from matched pairs of bellies. Initial comparisons were made on bacon sectioned into eighths and quarters. These sections were found to be too small for stitch pump convenience and uniform cure distribution.

Table I shows the compositional variables from four different experiments in which the matched pairs of bacon were sectioned into thirds. Data from the individual determinations were analyzed statistically by use of a split-plot analysis of variance (Snedecor and Cochran, 1974) with sides as the main plots and sections as the subplots. This analysis was performed for each of the compositional variables to determine which factors (side and/or section) had significant effects on these measures of composition. Composition was found to vary significantly ($p = 0.05$) from section to section (brisket, center, and flank) within the same side of bacon from a matched pair. The between-belly variation was significant at the $p = 0.05$ level for water, at the $p = 0.10$ level for protein, and not significant for fat. This could be due to the selection of bellies having comparable lean to fat ratios rather than more extreme variation. There is no significant difference for any of these compositional factors from side to side in the same section of a matched pair. These findings agree with those reported by Schroder and Rust (1974). In addition to the compositional variables, similar analyses for residual nitrite also showed the same behavior, i.e., significant ($p = 0.05$) variation from section to section within the same side and between sections of matched pairs, but no significant variation from side to side in the same section of a matched pair.

Data from six matched pairs were analyzed to determine the effect of side and section on nitrosamine formation; representative data are shown in Table II. A split-plot analysis of variance as used for the compositional factors indicated that all nitrosamine measurements were significantly ($p = 0.01$) different from one matched pair to the next and showed comparable results from section to section and side to side in the same matched pair.

The data from seven matched pairs of bellies were used to determine the correlation of the nitrosamine content in the edible portion, the drippings, and the total of the two with the compositional variables, added and residual nitrite (Table III).

The presence of both nitrosamines in the edible portion, drippings, and total is significantly correlated with the nitrite, residual and added, at the $p = 0.01$ level. The relationship to added nitrite is expected since higher residual nitrite usually occurs with higher added nitrite. The second most noticeable feature is the significant correlation ($p = 0.05$) between nitrosamines and compositional factors in the edible portions, whereas no correlation exists in the drippings. However, interpretation of correlations becomes extremely difficult for small concentrations of nitrosamines (<10 ppb) because the analytical variation is so large, i.e., ±3 ppb (Fiddler, 1978). This is true for two of the experiments in which the concentrations of both nitrosamines were low in the edible portion. It is necessary, therefore, to use the data for the drippings as well to obtain large enough values for meaningful computation. The highest correlation values and significance level were generally found in the total nitrosamine values (edible portion plus drippings). There is a weaker correlation between some nitrosamine values with fat, moisture, and protein content, which is not unexpected since these compositional factors are interrelated.

In summary, NPYR and NDMA are most highly correlated with residual and added nitrite and to a lesser degree with compositional factors. Therefore current efforts to reduce the level of nitrite used in the production of bacon coupled with the use of compounds such as ascorbate/erythorbate and α-tocopherol (Fiddler et al., 1978) could be expected to be effective in reducing nitrosamine

Table II. Nitrite and Nitrosamine Concentrations in Bacon from Matched Pairs of Bellies

| | | | brisket | | | | | center | | | | | flank | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | edible | | drippings | | | edible | | drippings | | | edible | | drippings | |
| pair | side | ANIT[a] | RNIT[a] | NDMA | NPYR[b] | NDMA[b] | NPYR[b] | RNIT[a] | NDMA | NPYR[b] | NDMA[b] | NPYR[b] | RNIT[a] | NDMA | NPYR[b] | NDMA[a] | NPYR[a] |
| 1 | left | 261 | 63 | 2 | 4 | 6 | 17 | 56 | 6[b] | 8 | 15 | 13 | 109 | 2 | 10 | 11 | 24 |
| | right | 239 | 52 | 2 | 8 | 8 | 28 | 61 | 2 | 2[c] | 7 | 21 | 66 | 2 | 7 | 6 | 13 |
| 2 | left | 229 | 156 | 2 | 11 | 11 | 41 | 129 | 5 | 32 | 23 | 43 | 156 | 4 | 20 | 12 | 37 |
| | right | 229 | 142 | 3 | 19 | 20 | 41 | 133 | 4[b] | 6 | 13 | 49 | 169 | 2 | 6 | 13 | 48 |
| 3 | left | 275 | 200 | 8[b] | 33 | 13 | 55 | 163 | 9[b] | 45 | 28 | 47 | 196 | 6[b] | 29 | 34 | 51 |
| | right | 219 | 131 | 7[b] | 36 | 25 | 39 | 157 | 6[b] | 41 | 25 | 42 | 188 | 4 | 38 | 28 | 49 |
| 4 | left | 169 | 36 | 2 | 7 | 2[c] | 6 | 26 | 3 | 15 | 2[c] | 7 | 22 | 3 | 10 | 2[c] | 5 |
| | right | 170 | 28 | 2 | 8 | 4 | 17 | 24 | 3 | 12 | 5 | 17 | 20 | 2 | 9 | 3 | 10 |

[a] NIT, $NaNO_2$ (ppm): A, added; R, residual. [b] Confirmed by mass spectrometry. [c] Not confirmed nitrosamines, ppb; corrected for recovery of internal standard nitrosamines.

Table III. Correlation ($r$ Values) between Nitrosamines and Bacon Composition

| | comp. var | | | | |
|---|---|---|---|---|---|
| | | | | sodium nitrite | |
| NA[a] | fat | moisture | protein | added | residual |
| ENDMA | 0.366[b] [b] | −0.365[b] | −0.400[a] | 0.469[a] | 0.537[a] |
| DNDMA | 0.221 | −0.172 | −0.281 | 0.588[a] | 0.688[a] |
| TNDMA | 0.283 | −0.240 | −0.344[b] | 0.630[a] | 0.734[a] |
| ENPYR | 0.334[b] | −0.349[b] | −0.469[a] | 0.514[a] | 0.650[a] |
| DNPYR | 0.204 | −0.124 | −0.313[b] | 0.625[a] | 0.766[a] |
| TNPYR | 0.307[b] | −0.253 | −0.432[a] | 0.613[a] | 0.758[a] |

[a] Nitrosamine in E, edible portion; D, drippings; T, total of edible portion plus drippings. [b] 48 degrees of freedom: superscript a, $p = 0.01\ |r| \geq 0.370$; superscript b, $p = 0.05\ |r| \geq 0.288$ (Snedecor and Cochran, 1974).

formation to minimum confirmable levels.


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